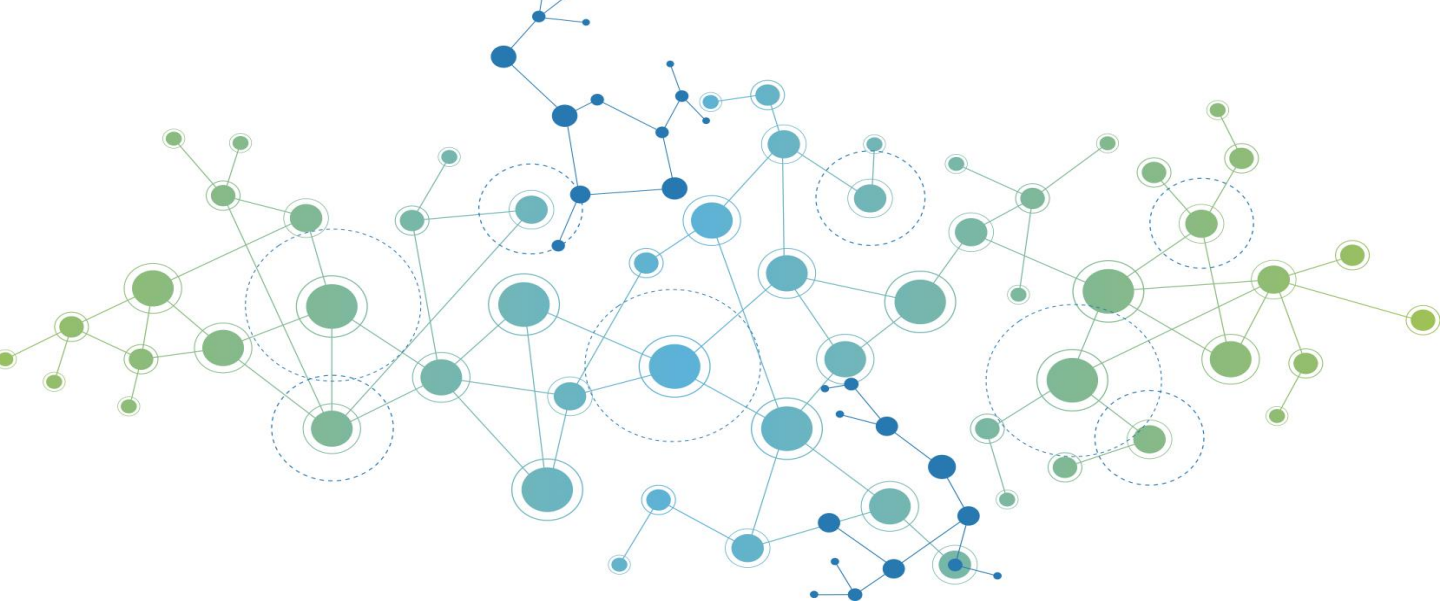


Proficiency testing toward reliable diagnosis

A network approach - strengthening veterinary diagnostics

Gemma Carlile | October 2019

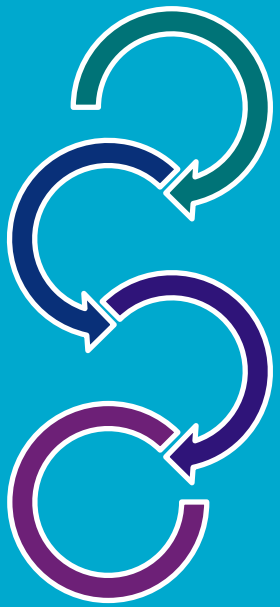
Regional PT programme 2011 to present

- The EQA program has involved a targeted approach to enable harmonized detection and response to emerging infectious diseases

- Building Regional Capacity of the National Laboratories for key Regional Diseases through external quality assurance.
  - Strengthen diagnosis capacity
  - Assure the quality of laboratory services

PT Objectives in Asia

- Building Regional Capacity of the National Laboratories for key Regional Diseases
- Use PT to access test optimisation (whole assay approach)
- Assess laboratory quality assurance e.g. processes followed, controls are used
- Compare tests in use across the region against relevant contemporary isolates from the region
- PT panel is designed to assess the sensitivity and specificity achieved by each participating laboratory for molecular detection using PCR



PT in Action

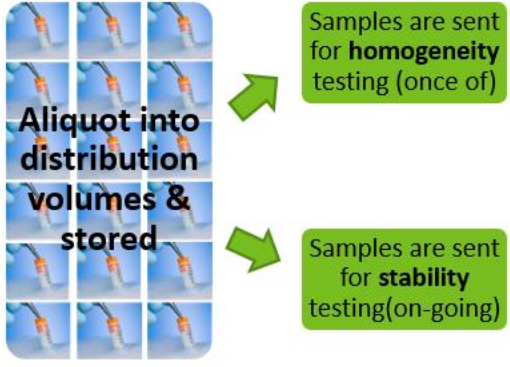
- PT helps to ensure new tests have been implemented correctly
  - **Training conducted in 2013 for implementation of ASF PCR diagnostics in SEA.**
- Success has been seen with development of regional SOP in use across the network
  - Adoption of the OIE method – **King *et. al.,* 2003**.

• Quality control material is provided with PT activities

PT Panel Composition

How do we prepare and assess samples for use in PT?

- PT involves performing the same test on the same samples and comparing results.
- Key requirement:
  - Samples are homogenous
  - Stable and
  - Suitable



Homogeneity results and all PRE and POST PT testing are recorded in a progressive record as part of our Quality Assurance system.

Acceptance criteria: mean Ct coefficient of variation <5%.

PT report review

How are participants assessed?

- Each laboratory is assessed based on agreement with the qualitative values assigned to each sample in the panel.
- Laboratory/assay performance is assessed as either – Acceptable or Unacceptable
- Where laboratories use real-time PCR additional analysis performed

Table 1 Test panel identity for swine disease samples, Asia Pacific Regional PT 2019

Sample	Virus ID	Diluents	Isolate	CSF	PRRS NA	PRRS EU	ASF	SIV
1	ASF	pig sera	Georgia 2007 (Genotype II)	negative	negative	negative	positive	negative
2	CSF	pig sera	Germany/1964 (Sub-genotype 1.1)	positive	negative	negative	negative	negative
3	ASF	pig sera	Malawi Lil-20/1 (1983) (Genotype VIII)	negative	negative	negative	positive	negative
4	PRRS-EU	pig sera	PRRS European Strain - Lelystad	negative	negative	positive	negative	negative
5	CSF	pig sera	Germany/1964 (Sub-genotype 1.1)	positive	negative	negative	negative	negative
6	PRRS-NA	pig sera	PRRS - strain NADC-8	negative	positive	negative	negative	negative
7	ASF	pig sera	Malawi Lil-20/1 (1983) (Genotype VIII)	negative	negative	negative	positive	negative
8	negative	pig sera	Negative pig sera	negative	negative	negative	negative	negative
9	PRRS-EU	pig sera	PRRS European Strain - Lelystad	negative	negative	positive	negative	negative
10	ASF	pig sera	Malawi Lil-20/1 (1983) (Genotype VIII)	negative	negative	negative	positive	negative
11	SIV	allantoic fluid	A/Swine/Pinjarra/AS-11-1723-3/2011 pH1N1	negative	negative	negative	negative	positive
12	ASF	pig sera	BA71V (Genotype 1)	negative	negative	negative	positive	negative
13	ASF	pig sera	Georgia 2007 (Genotype II)	negative	negative	negative	positive	negative
14	PRRS-SEA	pig sera	PRRS-SEA Circulating strain	negative	positive	negative	negative	negative
15	PRRS-NA	pig sera	PRRS - strain NADC-8	negative	positive	negative	negative	negative
16	ASF	pig sera	BA71V (Genotype 1)	negative	negative	negative	positive	negative
17	SIV	allantoic fluid	A/Swine/Pinjarra/AS-11-1723-3/2011 pH1N1	negative	negative	negative	negative	positive
18	PEDV	pig sera	PEDV Colorado	negative	negative	negative	negative	negative

What to assess?

- There are 2 main sources of variability in the results for PT:
  - variation between laboratories and
  - variation within laboratory
- The aim during analysis is to evaluate and provide feedback on both of these types of variation.
- In order to do this participants must perform the same testing on the same test item.
- The program is designed so that pairs of related results are obtained – split sample pairs or uniform sample pairs

Analysis of real-time results using related samples

Statistic	Ct values: Sample 7	Ct values: Sample 10
No. of Results	17	17
Median	32.57	32.60
Normalised IQR	1.79	2.24
Robust CV	5%	7%
Minimum	26.26	26.49
Maximum	36.80	35.42
Range	10.55	8.93

- Statistical analysis is performed on either split or uniform related samples
- Laboratory must report detection of each sample and provide a Ct value to be included in statistical analysis

Analysis of real-time results using related samples

Laboratory	Results		Between-Laboratory	Within-Laboratory
	Ct values: Sample 7	Ct values: Sample 10	Z-Score	Z-Score
A1	32.0	32.2	-0.21	0.28
AD1	33.7	33.4	0.43	0.38
AF1	30.4	30.9	-0.87	0.66
AG1	35.3	34.5	1.06	1.12
AH1	33.4	34.1	0.51	0.96
E1	32.6	32.6	0.00	0.03
F1	36.8	31.4	0.69	7.44 §
G1	35.1	33.4	0.77	2.29
M1	31.3	31.2	-0.60	0.25
N1	33.5	35.2	0.81	2.35
O1	31.9	33.0	-0.07	1.55
R1	36.2	35.4	1.47	1.11
S1	30.0	29.9	-1.22	0.15
U1	32.9	34.6	0.54	2.33
V1	26.3	26.5	-2.82	0.32
W1	29.5	29.6	-1.39	0.17
AK1	32.5	32.5	-0.05	0.02

The between-laboratories and within-laboratory Z-scores are for the related pair, samples 7 and 10. § denotes an outlier, i.e. |z-score| ≥± 3

- **Between laboratory z-score** compares a laboratory's results to the group median
- **Within Laboratory z-score** assesses the difference in Ct values reported by each laboratory
- A z-score of ≥3 is an outlier
- The data is provided in a table and in graphical formats

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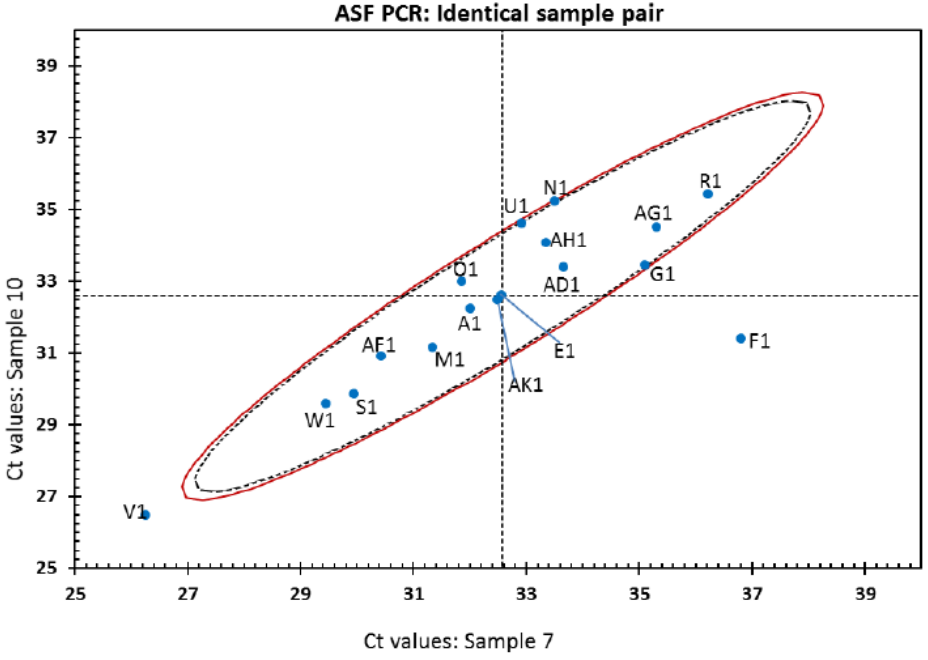
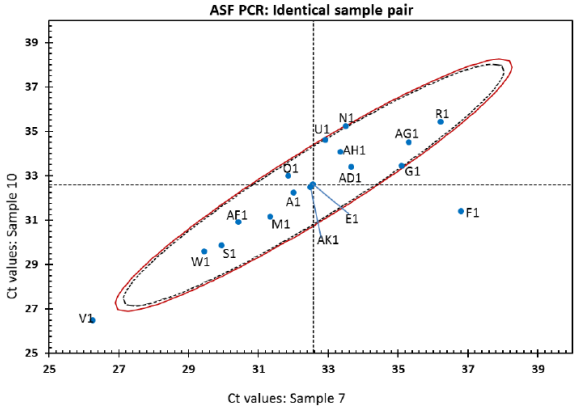
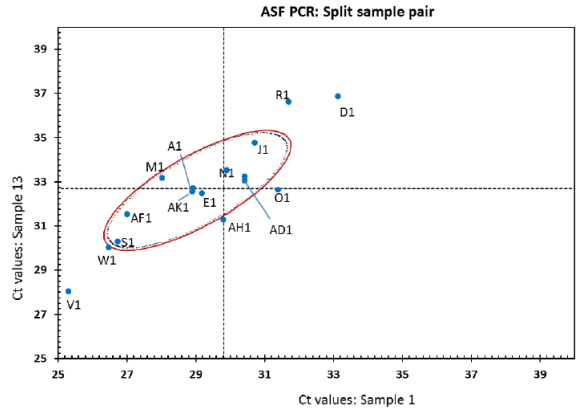
Analysis of real-time results using related samples



- The Youden plot maps the Ct values for each laboratory for the sample pair analysed
- The ellipse surrounding the data points defines the 99th – percentile boundary
- The dotted lines intersecting the axes indicate the median Ct values for each sample
- Results that plot outside the ellipse may result in an observation or condition in the assessment of the laboratory

Analysis of real-time results using related samples





- The shape of the Youden plot will change depending on:
  - The number of participants (minimum of 4 required in order to do statistical analysis),
  - How skewed the data is
  - The range of results for each sample

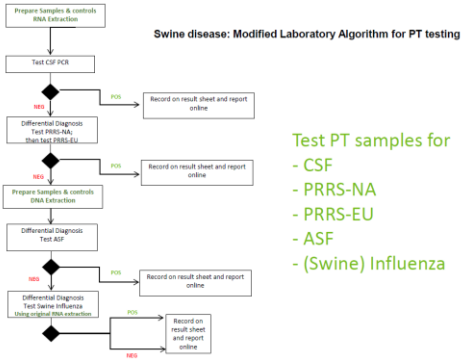
Regional PT program for swine diseases

SEA			SA & SEA			
2011	2012	2013	2014	2015	2016/2017	2018
CSF PCR	CSF PCR	CSF PCR	CSF PCR	Swine Disease	Swine Disease	Swine Disease
PRRS PCR	PRRS PCR	PRRS PCR	PRRS PCR	CSF PCR ASF PCR PRRS PCR	CSF PCR ASF PCR PRRS PCR	CSF PCR ASF PCR PRRS PCR
	ASF PCR	ASF PCR	ASF PCR	Influenza A PCR	Influenza A PCR	Influenza A PCR

2012-2014 single target panel for ASF

2015-2019 swine disease panel (CSF/PRRS/ASF/SIV)

Regional PT panels 2019



Test PT samples for
- CSF
- PRRS-NA
- PRRS-EU
- ASF
- (Swine) Influenza

- Panel Descriptions;
  - **Avian diseases** – Matrix PCR and APMV-1 (ND) PCR
    1. To include Influenza A relevant and circulating viruses
    2. To include Newcastle disease virus (NDV) class II, circulating field isolates

       and vaccine strains
  - **Swine diseases**
    1. To include CSF, PRRS (NA & EU), ASF and swine influenza viruses and differentials
    2. The swine diseases PCR panel for 2019 proficiency testing consisted of 18 samples

- Samples to be tested by Laboratories standard diagnostic approach - **Dx Algorithm** could be applied.

Swine Diseases Regional PT

- Panel includes ASF, CSF, PRRS, Swine Influenza (and negative) samples
- In 2019 – 28 laboratories from 20 countries enrolled (24 Labs supported by FAO, 3 labs supported by OIE)
  - 25 laboratories submitted results
- Represents a ~doubling in participation in 2019 up from 12 to 22 labs

2019 Participation

South East Asia

- Cambodia
- Indonesia (x4)
- Laos
- Malaysia
- Myanmar (x2)
- Philippines
- Thailand (x5)
- Vietnam (x3)

South Asia

- Bangladesh (x2)
- Bhutan
- India
- Nepal
- Sri Lanka

Central/East Asia

- Mongolia
- Chinese Taipei
- China (x2)

Pacific

- New Caledonia

10
ASEAN countries

5
SAARC countries

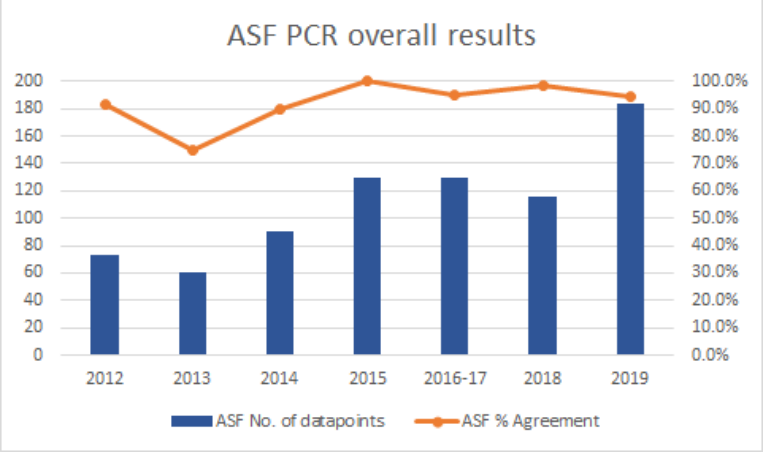
4
Central/East Asian and Pacific countries

76%
Avian disease panel participation (19/25)

89%
Swine disease panel participation (25/28)

Swine Disease PT - ASF PCR

- 15/18 laboratories utilised the method by King et al., 2003.
- Other methods used include:
  - Zsak et al., 2005
  - Unpublished methodologies x 2
  - Some performed parallel conventional PCR by Aguero et al 2003.
- Magnetic bead-based extraction and column used - did not correlate to the sensitivity of detection of ASF in the panel.



Swine disease results

CSF

- 20 submissions
- 6 qPCR and 3 conventional methods
- 100% agreement for all real-time PCR results.

SIV

- 18 submissions
- 3 qPCR and 2 conventional methods
- 100% agreement for all real-time PCR results

PRRS – NA strains

- 20 submissions
- 5 qPCR and 3 conventional methods
- 80% agreement

PRRS – EU strain

- 18 submissions
- 4 qPCR and 3 conventional methods
- 75% agreement

The common/most important issues identified

- Common causes of an ‘Unacceptable’ assessment were:
  - Failing to detect a positive sample (lack of sensitivity)
  - Reporting a negative sample as positive (Sample mis-handling and/or sample contamination)
  - Wrong interpretation of data and failure of authorisation procedures (a positive gel band or valid Ct result being reported as negative)
  - Common cause of an ‘Acceptable with condition’ assessment was an outlying z-score indicating lack of sensitivity or repeatability identified (labs must review procedures)

Value add activities - ‘backstopping’

- PT is complemented by backstopping missions – **critical** to assist, advise and troubleshoot identified problems - **involves all laboratory staff**

- Scientists with expertise in a range of diagnostic techniques travel to participating laboratories to;
  - discuss PT results,
  - provide technical advice in a range of areas,
  - assess diagnostic laboratory spaces and practices, (e.g. biosafety, quality assurance and documentation).

BACKSTOPPING MISSIONS – 2018/2019

- Targeted laboratories who participated in PT – 14 labs
    - Indonesia x 4 DICs
    - Philippines
    - Malaysia
    - Vietnam x 1 lab
    - Brunei
    - Bangladesh x 2 labs
    - Sri Lanka
    - Nepal
    - Bhutan
    - India

Food and Agriculture Organization of the United Nations

Value add activities - ‘backstopping’

- The long-term goals are:
  - to assist laboratories and institutes in their transition to accreditation;
  - enable regional centres of excellence to conduct PT for their own satellite laboratories and for the region.

Laboratory services have been strengthened though an iterative process of monitoring, evaluating, reflecting and learning

Thank you

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World Organisation for Animal Health

Food and Agriculture Organization of the United Nations

CSIRO